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Effect of Age on Sensitivity of 'Daphnia magna' to Cadmium, Copper and Cyanazine

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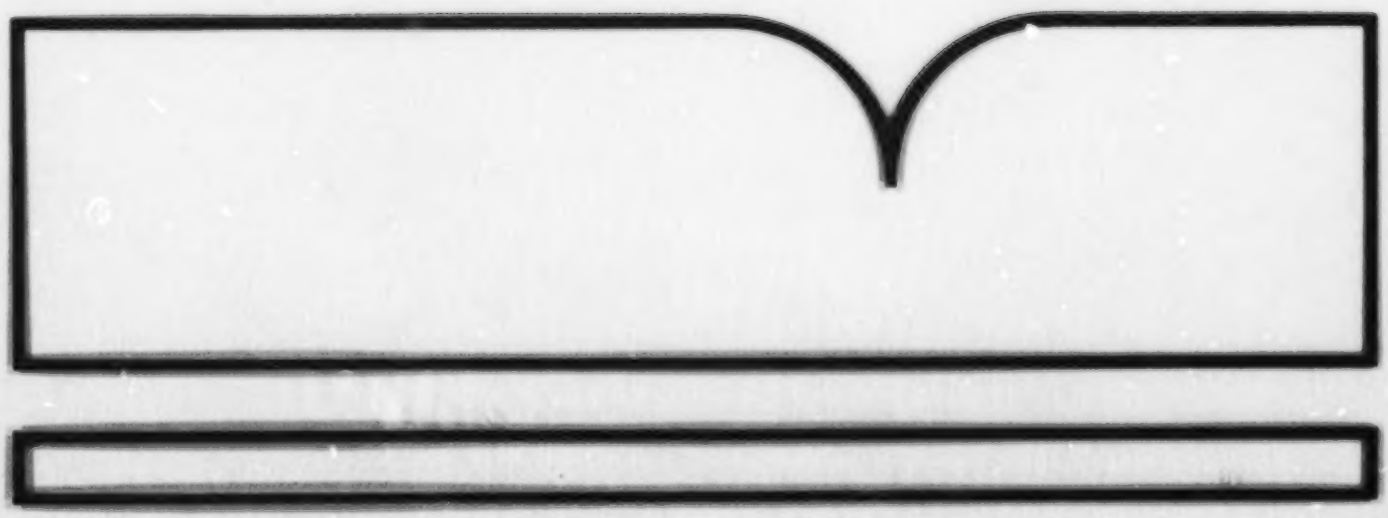

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Short Communication

EFFECT OF AGE ON SENSITIVITY OF *DAPHNIA MAGNA* TO CADMIUM, COPPER AND CYANAZINE

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Abstract—*Daphnia magna* were exposed to cadmium, copper and cyanazine to determine the relative sensitivities of several age groups: less than 4 h, less than 24 h, 1 d, 2 d, 3 d, 4 d, 5 d and 6 d old. Mean cadmium 48-h EC50 values for each age group ranged from 23 to 164 μg/L. Mean copper EC50 values ranged from 6 to 18 μg/L. Cyanazine EC50 values ranged from 53 to 106 mg/L. The 1-d-old and 5-d-old *Daphnia* mean EC50s were 48 and 49 μg/L for cadmium, 10 and 10 μg/L for copper and 84 and 86 μg/L for cyanazine, respectively. These similar sensitivities indicate that older animals can be used in tests equally as well as younger animals, thus simplifying the recovery of daphnids in acute sediment toxicity tests.

Keywords—*Daphnia magna* Toxicity Cadmium Copper Cyanazine Sediment

INTRODUCTION

A study was conducted to determine if the sensitivity of older and larger *Daphnia magna* juveniles to toxic materials was similar to that of the animals less than 24 h old, which are recommended for routine acute 48-h bioassays. Because *Daphnia* are difficult to recover from sediment samples after testing is completed, larger *Daphnia* are needed as test animals in screening potentially toxic sediment samples. For the sediment test [1], 800 ml water is added to a 200-ml sediment sample in a 1,000-ml beaker, and *Daphnia* are placed in the beaker. After 48 h of exposure with aeration, the daphnids are recovered by pouring the water and the fine suspended sediment (but not the bulk of the sediment) in the beaker through a fine-mesh screen that retains the *Daphnia*. The animals are transferred from the screen to clean water for counting. If larger, 5-d-old *Daphnia* were as sensitive as the animals less than 24 h old, they could be used, and the recovery of test animals would be greatly simplified.

D. magna are very sensitive to toxicants and are the freshwater aquatic test animal most frequently used in toxicity studies. Adema [2] showed that 1-d-old and 7-d-old *Daphnia* had the same sensitivities to trichloroethane, dieldrin and pentachlorophenol, but 1-d-old animals were significantly more sensitive to dichloroaniline. In tests conducted by Cairns et al. [3] and Nebeker et al. [1], the sensitivities of several species of invertebrates were compared using sediments from contaminated locations in the field and spiked control sediments. In 48-h tests, *D. magna* were found to be more sensitive than the following species in 10-d tests: the midge *Chironomus tentans*, the annelid *Lumbriculus variegatus*, the crustaceans *Gammarus lacustris* and *Hyalella azteca* and the mayfly *Hexagenia limbata*. Based on these results, we are expanding the use of *D. magna* in sediment acute bioassays (and chronic tests), and plan to use larger *Daphnia* if possible.

METHODS

The methods used for the *Daphnia* exposures were variable, as the impact of several parameters (water hardness, beaker size, food, number of animals) on 48-h EC-50 values (50% immobility at 48 h) was investigated. Procedures outlined by the American Society for Testing and Materials [4] were used as guidelines. Ten, fifteen or twenty animals of known age (less than 4 h, less than 24 h, 1 d, 2 d, 3 d, 4 d, 5 d and 6 d old) were placed in each of two 250- or 1,000-ml beakers at each of five or more concentrations of the toxicant. The

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temperature was held at 20°C; the photoperiod was 15 h of light and 9 h of darkness. A 48-h EC50 value, based on mean measured chemical values, was calculated (with 95% confidence limits) for each age group using the Trimmed Spearman-Karber method [5]. The EC50 values and their confidence limits for the age groups were then compared to determine whether differences were significant. The EC50 values were not significantly different if the 95% confidence limits overlapped.

Daphnia were reared in continuous culture in eight 4-liter jars, with approximately 60 *Daphnia* per jar. The *Daphnia* were kept for 4 weeks and discarded. Two new jars were started each week to maintain continuous cultures. Culture water (well water with additional chemicals added: $NaHCO_3$, $CaSO_4$, $MgSO_4$, KCl) was maintained at a total hardness of 200 mg/L (as $CaCO_3$) and was changed three times each week (Monday, Wednesday, Friday). On those days the *Daphnia* were fed 2.0 mg/L (dry weight) of the alga *Selenastrum capricornutum* plus 5 mg/L (dry solids) fish food solution [6]. On Tuesdays and Thursdays, the *Daphnia* were given 1.0 mg/L algae (which maintained a light green tint to the water in the jars). The cultures were maintained at 19.5 ± 0.5°C; the photoperiod was 15 h of light and 9 h of darkness. *Daphnia* started producing young when they were 8 to 10 d old. The different age groups needed for the tests were obtained by screening (retaining adults and discarding young) early in the morning and then waiting for new young to be released. The young were then screened from the adults and held in separate jars until they attained test age. They were fed and changed on the same schedule used for the adults. One molt occurs during the 48-h exposure of 5-d-old *Daphnia*, which ensured that the sensitive molting period was included in the test.

All test water was obtained from a well. Hardness (32 to 76 mg/L as $CaCO_3$), pH (6.8 to 7.8) and alkalinity (31 to 69 mg/L), which were constant during any one test, were monitored using the standard methods outlined by the American Public Health Association [7] and the U.S. Environmental Protection Agency (EPA) [8]. Reagent-grade cadmium chloride, copper chloride and the herbicide cyanazine were used for testing. Stock solutions were prepared by dissolving copper and cadmium in acidified (0.1 ml HNO_3/L), deionized water. Metal concentrations were analyzed on a Perkin-Elmer 305b atomic absorption spectrophotometer following EPA-approved quality assurance procedures [7,8]. Cyanazine stock solution (160 mg/L) was prepared by saturating deionized water with reagent-grade cyanazine over a 4-d period. Cyanazine concentrations in water were determined by liquid-liquid extraction with ethyl acetate and by analysis of the extracts with nitrogen-phosphorus flame-ionization gas chromatography. Cyanazine spike recovery averaged 97%, with duplicate analyses of test concentrations averaging ±4% variation.

RESULTS AND DISCUSSION

Daphnia mean 48-h EC50 values for cadmium ranged from 23 μg/L for 6-d-old daphnids to 164 μg/L for 2-d-old animals (Table 1). Tests with different age groups, conducted at different water hardnesses (32 to 76 mg/L as $CaCO_3$), with and without food and in two beaker sizes resulted in a wide range of EC50 values (Table 2). The very young animals (less than 4 h old) were relatively tolerant, with a mean EC50 value of 109 μg/L Cd. Generally, it is expected that softer water, larger containers (more volume/animal) and no food produce lower EC50 values, but in our tests this was not always the case. The EC50 values often were not significantly different ($\alpha = 0.05$). The less-than-24-h-old and 5-d-old *Daphnia* mean cadmium EC50 values were 46 and 49 μg/L, respectively, showing that animals of either age could represent the species in acute toxicity tests. The younger age groups were somewhat more sensitive, but the confidence limits of the less-than-24-h-old and 5-d-old animals (and most others also) overlapped, indicating that the EC50 values were not significantly different.

Mean EC50 values for copper (Table 3) ranged from 6 μg/L for 2-d-old daphnids to 18 μg/L for 6-d-old animals. The relatively small range of water hardness values (27 to 51 mg/L as $CaCO_3$) did not have a significant effect on the EC50 val-

Table 1. 48-h *Daphnia* age sensitivity to cadmium

Age	Mean EC50 (μg/L)	EC50 range (μg/L)	Number of tests
<4 h	109	59–178	8
<24 h	46	20–104	4
1 d	48	4–146	5
2 d	164	37–307	8
3 d	63	15–111	2
4 d	82	21–143	2
5 d	49	17–131	8
6 d	23	7– 40	2

Table 2. Variation in EC50 values for *Daphnia magna* age groups exposed to cadmium under different test conditions

Age group[a]	EC50 (μg/L Cd)	Beaker size (ml)	Water hardness (mg/L as $CaCO_3$)	No. of animals per beaker
<4 h	59(46–75)	250	76	10
<4 h	84(62–114)	250	74	10
<4 h	99(78–125)	250	41	10
<4 h	164(142–188)	250	38	10
<4 h	71(56–88)	1,000	76	10
<4 h	178(113–281)	1,000	74	10
<4 h	116(91–147)	1,000	74	10
<4 h	101(78–130)	1,000	71	15
1 d	4(3.5–5.2)	250	71	15
1 d	8(7–10)	250	41	20
1 d	64(53–77)	250	38[b]	10
1 d	16(12–22)	250	38	10
1 d	146(119–179)	1,000	74	10
2 d	55(24–124)	250	76	10
2 d	306(297–315)	250	74	10
2 d	98(75–127)	250	41	20
2 d	307(269–350)	250	38	10
2 d	37(27–51)	1,000	76	10
2 d	94(78–114)	1,000	74	10
2 d	277(187–412)	1,000	74	10
2 d	135(90–201)	1,000	71	15
5 d	17(—)	250	76	10
5 d	40(30–54)	250	74	10
5 d	30(22–41)	250	41	20
5 d	92(68–125)	250	38[b]	10
5 d	131(86–200)	250	38	10
5 d	25(12–54)	1,000	76	10
5 d	36(24–55)	1,000	74	10
5 d	18(14–24)	1,000	71	15

[a]Tests were not all conducted at the same time and place.
[b]Animals were fed. (Animals in other tests were not fed).

Table 3. 48-h *Daphnia* age sensitivity to copper

Age	Mean EC50 (μg/L)	EC50 range (μg/L)	Number of tests
<4 h	7	5–10	4
1 d	10	9–12	4
2 d	6	5–9	4
3 d	14	—	1
4 d	7	—	1
5 d	10	8–13	4
6 d	18	—	1

Table 4. 48-h *Daphnia* age sensitivity to cyanazine

Age	EC50 (mg/L)	Confidence limits
<4 h	106	88–127
1 d	84	65–107
2 d	53	42–66
3 d	95	75–120
4 d	93	75–114
5 d	86	40–183

Only one test was conducted with each age group.

ues. Five-day-old and 1-d-old *Daphnia* mean EC50 values were identical (10 μg/L). The reproducibility of the 19 copper tests, run under similar conditions, was excellent, and most confidence limits overlapped, indicating no significant differences between EC50 values.

The EC50 values for the six cyanazine tests were also similar, ranging from 53 mg/L for 2-d-old daphnids to 106 mg/L for animals less than 4 h old. The 1-d-old and 5-d-old animals (Table 4) had EC50 values of 84 and 86 mg/L, respectively, again indicating that the older animals can be used for 48-h screening tests to identify potentially toxic sediments.

The tests indicated no significant loss of sensitivity in older animals if daphnids of any age between 4 h and 6 d are used. Differences in sensitivity may relate to the number of molts per test as well as to the timing of molts during toxicant exposures. Many other variables, such as water chemistry, toxicant, temperature and dissolved oxygen level, are also important factors that may influence daphnid mortality. The apparently similar sensitivities of animals during the first week of life permits the use of older daphnids in tests in which their larger size would allow better recovery of live and dead organisms without sacrificing sensitivity.

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REFERENCES

1. **Nebeker, A.V., M.A. Cairns, J.H. Gakstatter, K.W. Malueg, G.S. Schuytema** and **D.F. Krawczyk.** 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. *Environ. Toxicol. Chem.* 3:617–630.
2. **Adema, D.M.M.** 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia* **59**:125–134.
3. **Cairns, M.A., A.V. Nebeker, J.H. Gakstatter** and **W.L. Griffis.** 1984. Toxicity of copper-spiked sedi-

ments to freshwater invertebrates. *Environ. Toxicol. Chem.* 3:435–445.

4. **American Society for Testing and Materials.** 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-80. Philadelphia, PA, pp. 1–25.
5. **Hamilton, M.A., R.C. Russo** and **R.V. Thurston.** 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**:714–719. Correction **12**:417 (1978).
6. **Goulden, C.E., R.M. Comotto, J.A. Hendrickson, Jr., L.L. Hornig** and **K.L. Johnson.** 1982. Procedures and recommendations for the culture and use of *Daphnia* in bioassay studies. STP 766. American Society for Testing and Materials, Philadelphia, PA, pp. 139–160.
7. **American Public Health Association, American Water Works Association** and **Water Pollution Control Federation.** 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. American Public Health Association, Washington, DC.
8. **U.S. Environmental Protection Agency.** 1979. Methods for chemical analysis of water and wastes. EPA-600/4-79-020. Cincinnati, OH, pp. 220.2-1, 213.2-1.

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